# BIOCHEMICAL AND HEMATOLOGICAL COMPONENTS OF LIVESTOCK AND POULTRY

## Part 3. Free Amino Acids and Amino Compounds in Delaine Sheep Serum

ARS-S-119
May 1976

AGRICULTURAL RESEARCH SERVICE • U.S. DEPARTMENT OF AGRICULTURE

# CONTENTS



# TABLES

# BIOCHEMICAL AND HEMATOLOGICAL COMPONENTS OF LIVESTOCK AND POULTRY

## Part 3. Free Amino Acids and Amino Compounds in Delaine Sheep Serum

By J. F. Cooper, F. M. Farr, H. R. Crookshank, and H. E. Smalley[1]

## ABSTRACT

Two trials were conducted to measure the levels of free amino acids and amino compounds in Delaine sheep serum. The 21 sheep in the first trial were fed a commercial feed supplement and hay, and the 18 sheep in the second trial were on grass pasture and a commercial supplement and hay. Significant variations in the levels of many free amino acid and amino compounds in mature wethers and rams were found in the first trial. In the second trial, arginine, 3-methylhistidine, and tyrosine were significantly greater in both male yearlings and male lambs than in mature wethers. Lamb serum had a higher level of 3-methylhistidine than yearling serum. Comparison of female sheep in trial 2 showed that lambs have a higher histidine and lower isoleucine level than either yearlings or mature ewes. Glycine, glutamic acid, and 1-methylhistidine were significantly higher in female yearlings and female lambs than in mature ewes. The level of glutamic acid was higher and 1-methylhistidine lower in female lambs than in female yearlings. **KEY WORDS: amino acids, amino compounds, Delaine sheep, Delaine sheep serum, livestock serum, serum, sheep.**

## INTRODUCTION

Changes in the levels of free amino acids in the plasma and urine of humans have been related to abnormalities such as uremia, renal disease, and congenital malformations.[2] Before similar irregularities can be detected and interpreted in animals, normal levels of free amino acids must be established. Our research established the normal levels of free amino acids and amino compounds in the blood serum of sheep fed a commercial feed supplement and hay and also normal levels in sheep that were on pasture plus a daily supplementary regulated amount of commercial mixture and hay.

## EXPERIMENTAL PROCEDURE

### Trial 1

Sixteen mature Delaine wethers and five mature Delaine rams that were confined as a group were fed 340 grams per head of a commercial

[1] Biological laboratory technician (biochemistry), biological laboratory technician (biochemistry), research chemist, and veterinary medical officer, Veterinary Toxicology and Entomology Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, College Station, Tex. 77840.

[2] Condon, J. R., and Asatoor, A. M. 1971. Amino acid metabolism in uremic patients. Clin. Chim. Acta 32: 333.

McGale, E. H. F., Pickford, J. C., and Aber, G. M. 1972. Quantitative changes in plasma amino acids in patients with renal disease. Clin. Chim. Acta 38: 395.

Roszkowski, I., Iwanska, J., Myszkowski, L., Brzeski, J., and Schmalhofer, A. 1970. Blood serum amino acids of nonpregnant women who have given birth to children with gross developmental malformations. Am. J. Obstet. Gynecol. 108: 1213.

feed supplement and 2 kilograms per head of alfalfa hay per day for 6 months (table 1). These animals were withdrawn from feed 12 hours before the initial bleeding. Four of the wethers were continued as above, additional samples being taken on the 9th, 15th, and 27th days after the initial bleeding to insure that data obtained from the bleedings could be used as normal levels. Blood samples were taken by jugular puncture, the blood was allowed to clot, and serum was obtained by double centrifugation of the blood. The serum was deproteinized with picric acid according to the Stein and Moore method[3] and analyzed for free amino acids[4] by using the Beckman model 121 amino acid analyzer interfaced with a digital integrator. Amino acids of known concentrations[5] were used as standards in order to identify and convert the samples to micromoles of amino acids per 100 milliliters of serum.

[3] Stein, W. H., and Moore, S. 1954. The free amino acids of human blood plasma. J. Biol. Chem. 211: 915.

[4] Moore, S., Spackman, D. H., and Stein, W. H. 1958. Chromatography of amino acids on sulfonated polystyrene resins: An improved system. Anal. Chem. 30: 1185.

Spackman, D. H., Stein, W. H., and Moore, S. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30: 1190.

TABLE 1.—*Amino acids in experimental diets*
[Trials 1 and 2]

| Amino acid or compound[1] | Percentage of amino acids in— | | |
|---|---|---|---|
| | Commercial feed supplement[2] | Alfalfa hay | Bermudagrass pasture |
| Lysine | 0.59 | 0.64 | 0.19 |
| Histidine | .34 | .19 | .06 |
| Ammonia | .95 | .68 | .10 |
| Arginine | 1.15 | .65 | .18 |
| Aspartic acid | 1.42 | 1.46 | .35 |
| Threonine | .50 | .56 | .14 |
| Serine | .63 | .54 | .15 |
| Glutamic acid | 2.65 | 1.32 | .45 |
| Proline | .65 | .60 | .13 |
| Glycine | 1.73 | .64 | .18 |
| Alanine | .75 | .71 | .21 |
| Valine | .72 | .68 | .18 |
| Methioine | .10 | .10 | .02 |
| Isoleucine | .52 | .56 | .14 |
| Leucine | 1.08 | .99 | .26 |
| Tyrosine | .51 | .45 | .10 |
| Phenylalanine | .77 | .65 | .16 |
| Protein | 17.55 | 19.72 | 4.00 |

[1] Determined by using a Beckman model 121 automatic amino acid analyzer. Samples were prepared according to the hydrolyzate procedure outlined in the instruction manual for the Beckman amino acid analyzer.

[2] Contained (g/100 g of mixture): corn chips, 29; crimped oats, 28; cottonseed meal, 20; alfalfa-leaf meal, 20; steamed bonemeal, 2; mixing salt, 1.

### Trial 2

Eighteen Delaine sheep (three mature wethers, two male and four female yearlings, three male and three female lambs, and three ewes) were on pasture (table 1) and fed the same amount of commercial mixture and alfalfa hay per day cited in trial 1. As in trial 1, these sheep were also allowed to remain on their diet for 6 months and were withdrawn from feed 12 hours before the initial bleeding. Six of the animals (one mature wether, two female yearlings, one male and one female lamb, and one ewe) continued to be fed the same diet and again were withdrawn from feed 12 hours before bleeding on the 2d and 57th days after the initial bleeding to insure that data obtained from the bleedings were comparable and could be used as normal levels. Samples were taken by jugular puncture and treated the same as in trial 1.

## RESULTS AND DISCUSSION

### Trial 1

A total of 38 free amino acids and amino compounds were identified in the blood serum of the sheep fed a commercial mixture and hay (table 2). Because there were no variations in the bleedings taken on the 9th, 15th, and 27th day after the initial bleeding, a one-way analysis of variance test was run on all bleedings. Results indicated that the levels of $\alpha$-aminoadipic acid, valine, isoleucine, leucine, ammonia, and carnosine were significantly greater in wethers than in rams and that the levels of phosphoserine, asparagine+glutamine, glycine, $\alpha$-amino-$n$-butyric acid, 1-methylhistidine, and histidine were greater in rams at the same level of significance. The levels of phenylalanine and taurine were significantly higher in wethers. At the highest level of significance ($P<0.05$), wether serum contained larger quantities of alanine, methionine, and lysine; ram serum contained more ethanolamine.

Because sheep of the same age were used in this experiment, differences in the levels of amino acids and amino compounds were probably due to sex.

[5] Hamilton Amino Acid Calibration Standards Type P–B and Type P–AN, Hamilton Co., Whittier, Calif.

## Trial 2

Levels of amino acids and amino compounds in the sheep that were fed the commercial mixture and hay were also determined by age for each sex (tables 3 and 4). Because there were no variations in the bleedings taken on the 2d and 57th days after the initial bleeding, statistical tests were run as in trial 1. When the male groups were compared with each other, lambs had significantly higher levels of proline, glutamic acid, 3-methylhistidine, serine, and tyrosine; yearlings had significantly higher levels of 1-methylhistidine and arginine; wethers had higher levels of taurine.

By comparing wethers with male yearlings, we found that wethers contained a higher level of taurine and that yearlings contained significantly higher levels of tyrosine, 3-methylhistidine, arginine, and 1-methylhistidine. Differences in the levels of serine, proline, and glutamic acid in the wethers and male yearlings were not significant.

Between wethers and male lambs, lambs produced significantly higher levels of certain amino acids: proline, glutamic acid, 3-methylhistidine, tyrosine, serine, and arginine. Differences in the levels of taurine and 1-methylhistidine in these animals were not significant.

Comparisons between male yearlings and lambs showed that yearlings had a higher level of 1-methylhistidine and that lambs had higher levels of taurine, glutamic acid, 3-methylhistidine, and proline. Differences between the levels of serine, tyrosine, and arginine in yearlings and lambs were not significant.

When the female groups were compared, significantly higher levels of proline, glutamic acid, histidine, 3-methylhistidine, and serine were detected in lambs. Yearlings produced more 1-methylhistidine, sarcosine, glycine, and isoleucine; ewes produced significantly more taurine and ornithine.

In a comparison with female yearlings, ewes contained significantly more taurine and ornithine. Yearlings had higher levels of 1-methylhistidine, glycine, and glutamic acid. Differences in the levels of serine, sarcosine, proline, isoleucine, histidine, and 3-methylhistidine in ewes and lambs were not significant.

Ewes had higher levels of sarcosine, isoleucine, and ornithine than female lambs. Lambs had significantly higher levels of proline, glutamic acid, histidine, 3-methylhistidine, serine, glycine, and 1-methylhistidine.

Comparisons between female yearlings and lambs showed that 1-methylhistidine was signifi-

*(Continued on page 6)*

TABLE 2.—*Free amino acids and amino compounds in serum of confined wethers and rams on commercial feed supplement and alfalfa hay, trial 1*

| Amino acid or compound | Micromoles of amino acid per 100 milliliters in— | | | |
|---|---|---|---|---|
| | Wethers (28 samples) | | Rams (5 samples) | |
| | Mean | Std. dev. | Mean | Std. dev. |
| Phosphoserine | 0.08 | 0.08 | 0.8 | [1]0.08 |
| Phosphoethanolamine | .5 | .5 | 1.3 | .6 |
| Taurine | 14.4 | [2]5.4 | 8.4 | 3.2 |
| Hydroxyproline | 3.0 | 1.4 | 2.8 | 2.0 |
| Aspartic acid | 1.0 | .4 | .7 | .07 |
| Threonine | 15.4 | 5.3 | 13.8 | 5.3 |
| Serine | 13.1 | 3.6 | 16.3 | 1.4 |
| Asparagine+glutamine | 28.3 | 8.0 | 67.5 | [1]7.9 |
| Sarconsine | 4.5 | 3.7 | 2.7 | 2.6 |
| Proline | 11.8 | 3.4 | 11.4 | 3.4 |
| Glutamic acid | 10.5 | 5.0 | 8.0 | 1.2 |
| Citrulline | 22.7 | 6.3 | 17.1 | 4.1 |
| Glycine | 56.6 | 8.8 | 101.5 | [1]12.4 |
| Alanine | 25.2 | [3]5.8 | 19.5 | 4.0 |
| $\alpha$-Aminoadipic acid | .3 | [1].2 | .04 | .04 |
| $\alpha$-Amino-$n$-butyric acid | .4 | .1 | .8 | [1].2 |
| Valine | 31.1 | [1]8.3 | 15.0 | 4.8 |
| Cystathionine | 1.4 | .3 | 1.3 | .2 |
| Methionine | 4.1 | [3].7 | 3.4 | .4 |
| Isoleucine | 11.2 | [1]2.6 | 7.3 | 2.0 |
| Leucine | 17.6 | [1]4.4 | 10.8 | 3.0 |
| Tyrosine | 8.0 | 1.9 | 6.2 | 2.1 |
| Phenylalanine | 6.4 | [4]1.5 | 4.0 | 1.1 |
| $\beta$-Alanine | .1 | .1 | 0 | 0 |
| $\beta$-Aminoisobutyric acid | 0 | ... | 0 | 0 |
| Hydroxylysine | ([5]) | ... | 0 | 0 |
| Allohydroxylysine | .05 | .05 | 0 | 0 |
| $\gamma$-Aminobutyric acid | .06 | .06 | 0 | 0 |
| Ornithine | 12.3 | 3.5 | 9.6 | 2.2 |
| Ethanolamine | 4.8 | 1.2 | 6.1 | [3]1.0 |
| Ammonia | 32.9 | [1]11.5 | 15.3 | 3.6 |
| Lysine | 14.3 | [3]4.9 | 9.2 | 2.6 |
| 1-Methylhistidine | 8.3 | 1.5 | 12.5 | [1]4.4 |
| Histidine | 4.1 | .8 | 6.8 | [1]1.2 |
| 3-Methylhistidine | 3.6 | 1.0 | 4.0 | 1.1 |
| Anserine | 9.1 | 1.7 | 8.3 | 1.6 |
| Carnosine | 4.3 | [1]1.6 | .8 | .5 |
| Arginine | 18.5 | 4.7 | 16.6 | 2.6 |

[1] Level of significance: $P<0.005$.
[2] Level of significance: $P<0.025$.
[3] Level of significance: $P<0.05$.
[4] Level of significance: $P<0.01$.
[5] Trace.

TABLE 3.—*Free amino acids and amino compounds in serum of male sheep on bermudagrass pasture plus commercial feed supplement and alfalfa hay, trial 2*

| Amino acid or compound | Micromoles of amino acid per 100 milliliters in— | | | | | |
|---|---|---|---|---|---|---|
| | Wethers (5 samples) | | Yearlings (2 samples) | | Lambs (5 samples) | |
| | Mean | Std. dev. | Mean | Std. dev. | Mean | Std. dev. |
| Phosphoserine | 0.2 | 0.2 | 0 | ... | 0.5 | 0.5 |
| Phosphoethanolamine | 0 | ... | 0 | ... | .1 | .1 |
| Taurine | 14.9 | [1 2]1.8 | 10.0 | 0.4 | 14.8 | [3]2.3 |
| Hydroxyproline | 1.1 | 1.1 | ([4]) | ... | 4.5 | 4.1 |
| Aspartic acid | 2.0 | 1.0 | 2.6 | .2 | 4.1 | 2.3 |
| Threonine | 11.2 | 1.8 | 12.5 | 1.7 | 24.3 | 12.9 |
| Serine | 10.9 | 1.6 | 11.6 | .7 | 17.1 | [5 6]4.5 |
| Asparagine+glutamine | 26.7 | 10.9 | 37.4 | 1.7 | 40.2 | 17.3 |
| Sarcosine | 12.3 | 3.2 | 16.5 | 5.3 | 13.8 | 8.8 |
| Proline | 12.0 | 1.0 | 11.9 | 1.7 | 28.6 | [7 8 9]8.1 |
| Glutamic acid | 11.2 | 3.7 | 14.9 | .2 | 25.1 | [3 7 8]4.5 |
| Citrulline | 19.3 | 3.4 | 23.4 | 2.7 | 28.1 | 6.7 |
| Glycine | 63.5 | 7.4 | 72.6 | 5.5 | 78.9 | 11.8 |
| Alanine | 26.2 | 3.1 | 23.3 | 1.5 | 30.9 | 4.5 |
| $\alpha$-Aminoadipic acid | .5 | .2 | .5 | .5 | 1.0 | .5 |
| $\alpha$-Amino-*n*-butyric acid | 1.0 | .6 | 1.2 | .07 | 1.0 | .4 |
| Valine | 29.3 | 3.5 | 26.2 | 1.9 | 27.7 | 7.3 |
| Cystathionine | .2 | .2 | 0 | ... | .1 | .1 |
| Methionine | 3.1 | .4 | 3.3 | .9 | 3.6 | 1.4 |
| Isoleucine | 9.9 | 1.2 | 11.4 | .5 | 9.1 | 1.9 |
| Leucine | 16.0 | 2.2 | 15.4 | .4 | 16.9 | 3.4 |
| Tyrosine | 7.0 | .6 | 9.5 | [10].2 | 11.4 | [5 11]2.8 |
| Phenylalanine | 5.7 | .8 | 6.0 | .07 | 8.0 | 2.0 |
| $\beta$-Alanine | .1 | .1 | ([4]) | ... | .4 | .4 |
| $\beta$-Aminoisobutyric acid | 0 | ... | 0 | ... | 0 | ... |
| Hydroxylysine | ([4]) | ... | 0 | ... | .2 | .2 |
| Allohydroxlysine | 0 | ... | 0 | ... | 0 | ... |
| $\gamma$-Aminobutyric acid | .6 | .6 | 0 | ... | .3 | .3 |
| Ornithine | 11.5 | 1.3 | 10.7 | .1 | 13.2 | 2.8 |
| Ethanolamine | 4.0 | 1.2 | 5.0 | 1.5 | 2.2 | 2.0 |
| Ammonia | 32.2 | 8.1 | 25.6 | 2.6 | 35.4 | 8.1 |
| Lysine | 16.0 | 1.3 | 17.0 | 1.4 | 19.3 | 2.4 |
| 1-Methylhistidine | 13.0 | 3.6 | 20.4 | [5 12 13].8 | 10.8 | 3.1 |
| Histidine | 5.5 | .8 | 6.6 | .2 | 7.3 | 1.6 |
| 3-Methylhistidine | 2.5 | .3 | 4.1 | [8 10].2 | 8.0 | [3 7]1.6 |
| Anserine | ([4]) | ... | ([4]) | ... | 1.7 | 2.4 |
| Carnosine | 2.8 | 1.3 | 1.7 | .1 | 3.1 | .8 |
| Arginine | 16.9 | 1.0 | 23.7 | [5 10]2.9 | 22.7 | [6]4.4 |

[1] $P<0.025$; comparison between wethers and yearlings.
[2] $P<0.05$; comparisons among wethers, yearlings, and lambs.
[3] $P<0.025$; comparison between yearlings and lambs.
[4] Trace.
[5] $P<0.025$; comparisons among wethers, yearlings, and lambs.
[6] $P<0.025$; comparison between wethers and lambs.
[7] $P<0.005$; comparisons among wethers, yearlings, and lambs.
[8] $P<0.005$; comparison between wethers and lambs.
[9] $P<0.05$; comparison between yearlings and lambs.
[10] $P<0.005$; comparison between wethers and yearlings.
[11] $P<0.01$; comparison between wethers and lambs.
[12] $P<0.01$; comparison between yearlings and lambs.
[13] $P<0.05$; comparison between wethers and yearlings.

TABLE 4.—*Free amino acids and amino compounds in serum of female sheep on bermudagrass pasture plus commercial feed supplement and alfalfa hay, trial 2*

| Amino acid or compound | Micromoles of amino acid per 100 milliliters in— | | | | | |
|---|---|---|---|---|---|---|
| | Ewes (5 samples) | | Yearlings (8 samples) | | Lambs (5 samples) | |
| | Mean | Std. dev. | Mean | Std. dev. | Mean | Std. dev. |
| Phosphoserine | 0.4 | 0.4 | 0.2 | 0.2 | 0.4 | 0.4 |
| Phosphoethanolamine | .2 | .2 | .1 | .1 | .2 | .2 |
| Taurine | 18.2 | [1 2]2.2 | 9.7 | 3.0 | 16.6 | [3]4.6 |
| Hydroxyproline | 2.5 | 2.5 | 6.1 | 5.9 | 3.8 | 3.5 |
| Aspartic acid | 1.4 | .9 | 2.0 | 1.1 | 2.7 | .9 |
| Threonine | 9.8 | 2.2 | 10.2 | 2.5 | 10.5 | 2.9 |
| Serine | 9.1 | 2.1 | 11.3 | 2.0 | 13.8 | [4 5]3.0 |
| Asparagine+glutamine | 24.0 | 5.9 | 30.9 | 9.8 | 35.4 | 13.0 |
| Sarcosine | 14.2 | [6]4.7 | 16.3 | [7 8]8.3 | 6.6 | 1.9 |
| Proline | 11.9 | 2.4 | 12.8 | 2.6 | 21.6 | [1 9 10]2.9 |
| Glutamic acid | 10.3 | 2.2 | 13.8 | [11]3.0 | 20.6 | [1 9 10]4.4 |
| Citrulline | 25.5 | 3.9 | 23.5 | 6.3 | 22.3 | 5.4 |
| Glycine | 49.5 | 11.0 | 72.4 | [8 12]17.0 | 70.0 | [5]11.6 |
| Alanine | 23.3 | 4.4 | 24.0 | 3.9 | 26.6 | 2.7 |
| $\alpha$-Aminoadipic acid | .9 | .7 | .4 | .2 | .8 | .2 |
| $\alpha$-Amino-$n$-butyric acid | .9 | .6 | .9 | .5 | .9 | .3 |
| Valine | 26.2 | 7.0 | 26.1 | 4.3 | 22.8 | 3.6 |
| Cystathionine | .2 | .2 | .3 | .3 | .2 | .2 |
| Methionine | 2.0 | .9 | 2.8 | .7 | 2.8 | .6 |
| Isoleucine | 9.5 | [5]8 | 10.2 | [7 8]1.5 | 8.0 | 1.0 |
| Leucine | 15.7 | .9 | 15.1 | 1.6 | 15.1 | 1.8 |
| Tyrosine | 7.1 | 1.3 | 7.2 | .8 | 8.1 | 1.1 |
| Phenylalanine | 6.1 | .5 | 6.4 | 1.0 | 6.7 | .8 |
| $\beta$-Alanine | .2 | .1 | .2 | .1 | .2 | .2 |
| $\beta$-Aminoisobutyric acid | 0 | ... | 0 | ... | 0 | ... |
| Hydroxylysine | .1 | .1 | .1 | .1 | .2 | .1 |
| Allohydroxylysine | 0 | ... | 0 | ... | 0 | ... |
| $\gamma$-Aminobutyric acid | .08 | .08 | .08 | .08 | 0 | ... |
| Ornithine | 15.1 | [4 5 12]2.4 | 11.1 | 2.6 | 12.0 | .7 |
| Ethanolamine | 2.1 | 2.1 | 2.8 | 2.4 | 2.5 | 2.3 |
| Ammonia | 25.6 | 4.8 | 35.7 | 13.3 | 38.3 | 13.8 |
| Lysine | 13.1 | 1.7 | 14.8 | 4.0 | 15.9 | .9 |
| 1-Methylhistidine | 8.0 | 2.6 | 18.8 | [1 2 3]4.3 | 11.6 | [13]1.5 |
| Histidine | 3.8 | 1.0 | 4.4 | .8 | 6.1 | [1 3 9].9 |
| 3-Methylhistidine | 3.9 | 1.6 | 3.9 | 1.6 | 7.9 | [1 9 10]1.1 |
| Anserine | 2.8 | 2.8 | 1.6 | 1.6 | 2.2 | 2.2 |
| Carnosine | 2.7 | 1.0 | 2.3 | 1.4 | 2.9 | .9 |
| Arginine | 15.6 | 3.1 | 16.8 | 2.4 | 19.6 | 2.4 |

[1] $P<0.005$; comparisons among ewes, yearlings, and lambs.
[2] $P<0.005$; comparison between ewes and yearlings.
[3] $P<0.01$; comparison between yearlings and lambs.
[4] $P<0.025$; comparisons among ewes, yearlings, and lambs.
[5] $P<0.025$; comparison between ewes and lambs.
[6] $P<0.01$; comparison between ewes and lambs.
[7] $P<0.025$; comparison between yearlings and lambs.
[8] $P<0.05$; comparisons among ewes, yearlings, and lambs.
[9] $P<0.005$; comparison between ewes and lambs.
[10] $P<0.005$; comparison between yearlings and lambs.
[11] $P<0.05$; comparison between ewes and yearlings.
[12] $P<0.025$; comparison between ewes and yearlings.
[13] $P<0.05$; comparison between ewes and lambs.

cantly higher in yearlings. Levels of sarcosine and isoleucine were also higher in yearlings. Levels of proline, glutamic acid, 3-methylhistidine, taurine, and histidine were higher in lambs. Differences between the levels of serine, glycine, and ornithine in yearlings and lambs were not significant.

Comparisons of mean and range values between wethers on feed and wethers on pasture and feed showed no significant differences.

## ACKNOWLEDGMENTS

The authors wish to acknowledge N. A. Durrant and R. G. Zboril, physical science aids, for their assistance in the preparation of samples.

*1976-G.P.O.-1750-S/671-583/59